

CLAIMS

1. A subtilase selected from the group consisting of
 - (a) a subtilase having an amino acid sequence which has at least 95% identity with the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2; and
 - (b) a subtilase which is encoded by a nucleic acid sequence which hybridizes under low stringency conditions with
 - (i) a complementary strand of the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1, or
 - (ii) a subsequence of (i) of at least 100 nucleotides.
2. A subtilase of claim 1 having an amino acid sequence which has at least 96% identity with the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2.
3. A subtilase of claim 2 having an amino acid sequence which has at least 97% identity with the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2.
4. A subtilase of claim 3 having an amino acid sequence which has at least 98% identity with the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2.
5. A subtilase of claim 4 having an amino acid sequence which has at least 99% identity with the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2.
6. A subtilase of claim 1, which comprises the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2.
7. A subtilase of claim 6, which consists of the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2.
8. A modified subtilase comprising a mutation of the amino acid sequence of amino acids 1 to 269 of SEQ ID NO:2 wherein the mutation comprises a substitution, deletion, and/or insertion of one or more amino acid residues.

9. A subtilase of claim 8, wherein the mutation comprises at least one modification in one of the positions 27, 36, 56, 76, 87, 96, 97, 98, 99, 100, 101, 103, 104, 120, 123, 129, 131, 132, 133, 143, 159, 167, 170, 192, 194, 206, 217, 218, 222, 224, 232, 235, 236, 245, 248, 252 and 274, wherein each position corresponds to the position of the amino acid sequence of the mature subtilisin BPN'.

10. A subtilase of claim 9, wherein the mutation comprises at least one modification selected from the group consisting of K27R, *36D, T56P, N76D, N87S, A97N, A98AT, A98AS, N99ND, N99NR, N99A, N99T, R101G, P103A, V104A, V104I, V104N, V104Y, D120H, N123S, P129K, P131H, A133P, A133D, A133E, T143K, *159D, *159E, Y167X, Y167A, R170X, R170S, A194P, Q206E, F217R, N218S, M222S, M222A, T224S, A232V, K235L, Q236H, Q245R, N248D, N252K and T274A.

11. A subtilase of claim 9, wherein the mutation comprises V104N+R101G, K27R+V104Y+N123S+T274A, N76D+V104A, R101G+P103A+V104I+*159D+A232V+Q236H+Q245R+N248D+N252K; or other combinations of the modifications K27R, N76D, R101G, P103A, V104I, V104N, V104A, V104Y, N123S, *159D, A232V, Q236H, Q245R, N248D, N252K and T274A.

12. A subtilase of claim 1, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with

(a) a complementary strand of the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1, or

(b) a subsequence of (i) of at least 100 nucleotides.

13. A subtilase of claim 12, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with

(a) a complementary strand of the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1, or

(b) a subsequence of (i) of at least 100 nucleotides.

14. A subtilase of claim 1, where the subtilase – when tested in the “Ovo-inhibition Assay” - has a residual activity of at least 15%.

15. A subtilase of claim 14, where the subtilase has a residual activity of at least 20%.
16. A subtilase of claim 15, where the subtilase has a residual activity of at least 25%.
17. A subtilase of claim 16, where the subtilase has a residual activity of at least 30%.
18. A subtilase of claim 17, where the subtilase has a residual activity of at least 35%.
19. A cleaning or detergent composition, comprising the subtilase of claim 1 and a surfactant.
20. A composition of claim 19, which additionally comprises an amylase, cellulase, cutinase, lipase, oxidoreductase, another protease, or mixture thereof.
21. A method for cleaning or washing a hard surface or laundry, the method comprising contacting the hard surface or the laundry with the composition of claim 19.
22. A method for removal of egg stains from a hard surface or from laundry, the method comprising contacting the egg stain-containing hard surface or the egg stain-containing laundry with the composition of claim 19.
23. An isolated nucleic acid sequence comprising a nucleic acid sequence which encodes for the subtilase of claim 1.
24. An isolated nucleic acid sequence encoding a subtilase, selected from the group consisting of
 - (a) a nucleic acid sequence having at least 85% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1; and
 - (b) a nucleic acid sequence which hybridizes under low stringency conditions with
 - (i) a complementary strand of the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1, or
 - (ii) a subsequence of (i) of at least 100 nucleotides.

25. A nucleic acid sequence of claim 24, having a nucleic acid sequence which has at least 86% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1

26. A nucleic acid sequence of claim 25, having a nucleic acid sequence which has at least 87% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

27. A nucleic acid sequence of claim 26, having a nucleic acid sequence which has at least 88% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

28. A nucleic acid sequence of claim 27, having a nucleic acid sequence which has at least 89% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

29. A nucleic acid sequence of claim 28, having a nucleic acid sequence which has at least 90% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

30. A nucleic acid sequence of claim 29, having a nucleic acid sequence which has at least 91% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

31. A nucleic acid sequence of claim 30, having a nucleic acid sequence which has at least 92% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

32. A nucleic acid sequence of claim 31, having a nucleic acid sequence which has at least 93% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

33. A nucleic acid sequence of claim 32, having a nucleic acid sequence which has at least 94% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

34. A nucleic acid sequence of claim 33, having a nucleic acid sequence which has at least 95% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

35. A nucleic acid sequence of claim 34, having a nucleic acid sequence which has at least 96% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

36. A nucleic acid sequence of claim 35, having a nucleic acid sequence which has at least 97% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

37. A nucleic acid sequence of claim 36, having a nucleic acid sequence which has at least 98% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

38. A nucleic acid sequence of claim 37, having a nucleic acid sequence which has at least 99% identity with the nucleic acid sequence of nucleotides 334 to 1140 of SEQ ID NO:1.

39. A nucleic acid construct comprising the nucleic acid sequence of claim 23 operably linked to one or more control sequences capable of directing the expression of the subtilase in a suitable host.

40. A recombinant expression vector comprising the nucleic acid construct of claim 39, a promoter, and transcriptional and translational stop signals.

41. A recombinant host cell comprising the nucleic acid construct of claim 39.

42. A host cell of claim 41, which is a bacterium.

43. A host cell of claim 42, wherein the bacterium is a *Bacillus*.

44. A host cell of claim 43, wherein the bacterium is a *B. clausii*.

45. A host cell of claim 41, which is a fungus or yeast.

46. A host cell of claim 45, which a filamentous fungus.

47. A host cell of claim 46, which an *Aspergillus*.

48. A method for producing the subtilase, comprising:

(a) cultivating a recombinant host cell of claim 41 under conditions conducive to the production of the subtilase; and

(b) recovering the subtilase.

49. A method for producing the subtilase of claim 1, comprising:
- (a) cultivating a *Bacillus* strain to produce a supernatant comprising the subtilase;
- and
- (b) recovering the subtilase.
50. The method of claim 49, wherein the strain is a *Bacillus clausii* strain.
51. The method of claim 50, wherein the strain is *Bacillus clausii* DSM 13585.